Thrombosis Research 127 Suppl. 3 (2011) S72–S75

Contents lists available at ScienceDirect

Thrombosis Research

journal homepage: www.elsevier.com/locate/thromres

New insights into the etiology of preeclampsia: identification of key elusive factors for the vascular complications

Asif Ahmed*

Gustav Born Centre for Vascular Biology, UoE/BHF Centre for Cardiovascular Sciences, Queen's Medical Research Institute, College of Medicine and Veterinary Medicine, University of Edinburgh, 47 Little France Crescent, Edinburgh, EH16 4TJ, United Kingdom

ARTICLE INFO

Keywords:
Preeclampsia
VEGF
sFlt-1
sEng
HO-1
CO

ABSTRACT

The incidence of preeclampsia is reduced by a third in smokers, but not in snuff users. Soluble Flt-1 (sFlt-1) and soluble endoglin (sEng) are increased prior to the clinical onset of preeclampsia. Animals exposed to high circulating levels of sFlt-1 and sEng elicit severe preeclampsia-like symptoms. Smokers have reduced circulating sFlt-1 and cigarette smoke extract decreases sFlt-1 release from placental villous explants. An anti-inflammatory enzyme, heme oxygenase-1 (HO-1) and its metabolite carbon monoxide (CO), inhibit sFlt-1 and sEng release. Women with preeclampsia exhale less CO than women with normal pregnancies and HO expression decreases as the severity of preeclampsia increases. In contrast, sFlt-1 levels increase with increasing severity. More importantly, chorionic villous sampling from women at eleven weeks gestation shows that HO-1 mRNA expression is decreased in women who go on to develop preeclampsia. Collectively, these facts provide compelling evidence to support the proposition that the pathogenesis of preeclampsia is largely due to loss of HO activity. This results in an increase in inflammation and excessive elevation of the two key anti-angiogenic factors responsible for the clinical signs of preeclampsia. These findings provide strong evidence for a protective role of HO-1 in pregnancy and identify HO as a target for the treatment of preeclampsia. The cardiovascular drugs, statins, stimulate HO-1 expression and inhibit sFlt-1 release *in vivo* and *in vitro*, thus, they have the potential to ameliorate early onset preeclampsia. The StAmP trial is underway to address this and if positive, its outcome will lead to the very first therapeutic intervention to prolong affected pregnancies.

Introduction

The ancient civilisations of China, Egypt and India had all documented the signs of eclampsia, the extreme consequence of unmanaged preeclampsia. The two key signs of preeclampsia, proteinurea and hypertension seen in the third trimester, were finally discovered during the mid to late nineteen-century. During this period the recognition grew that delivery resolved the shocking spectre of convulsions in pregnancy. Even today, the only effective therapy is delivery of the baby and the placenta. Early delivery often has serious consequences for the health of the baby, especially before 32 weeks gestation, whereas watchful waiting often employed to allow for fetal lung maturity, *in utero*, increases maternal risks. This review outlines a new mechanism of preeclampsia and presents the scientific basis of therapies for preeclampsia.

Preeclampsia is characterised by widespread endothelial damage, which leads to proteinurea and hypertension. The loss of endothelial nitric oxide synthase (eNOS) activity promotes endothelial dysfunction due to the "uncoupling" of eNOS resulting in the production of superoxide instead of nitric oxide (NO) [1]. Vascular endothelial growth factor (VEGF) is critical for homeostasis [2] and activates both VEGF receptor-1 (VEGFR-1) and VEGFR-2 to stimulate NO required for angiogenesis [3]. Indeed, one of the beneficial effects of statins in the prevention of cardiovascular events is their ability to enhance endothelial function by increasing eNOS expression and activity [4]. However, statins are contraindicated in pregnancy because of their potential teratogenic effects and until recently there were no strong scientific rationale for their use.

Have the culprits been identified?

The argument first articulated that preeclampsia may arise due to loss of VEGF activity and by the possible elevation of soluble Flt-1 (sFlt-1) [5] gained momentum when it was shown that adenoviral

* Correspondence: Professor Asif Ahmed, UoE/BHF Centre for Cardiovascular Sciences, Queen's Medical Research Institute, College of Medicine and Veterinary Medicine, University of Edinburgh, 47 Little France Crescent, Edinburgh, EH16 4TJ, United Kingdom.
Tel.: +44 131 242 9217; fax: +44 131 242 6779.
E-mail address: a.s.ahmed@ed.ac.uk (A. Ahmed).

0049-3848 /$ – see front matter

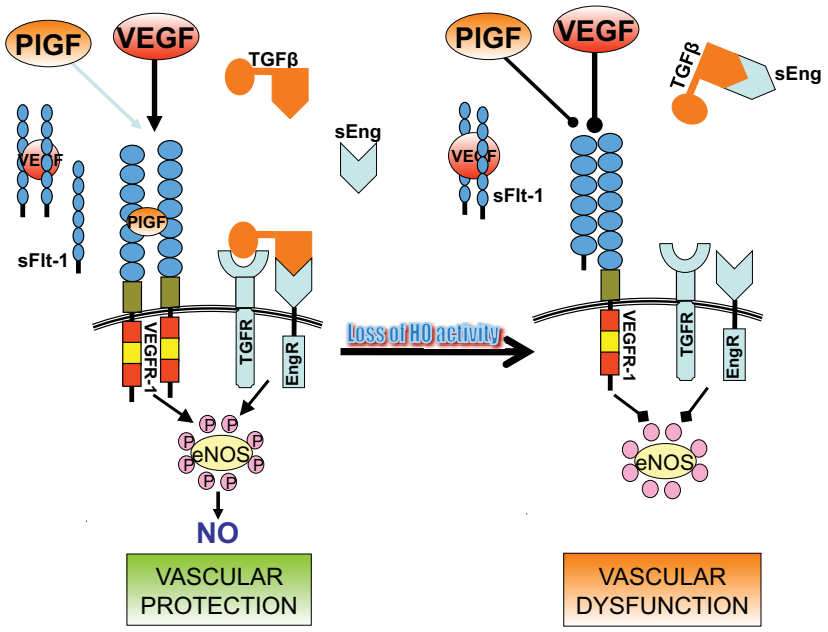

Fig. 1. Vascular dysfunction in preeclampsia: a diagrammatic representation. A functional endothelial monolayer requires VEGF, PLGF and TGFβ for normal endothelial function via activation of nitric oxide (NO). In preeclampsia VEGF protective signal is compromised due to an excess of soluble Flt-1 (sFlt-1), which is compounded by a decrease in the expression of PlGF and a rise in circulating soluble Eng (sEng).

overexpression of sFlt-1 to pregnant rats mimicked the clinical manifestations of preeclampsia [6]. This idea received further support as it became apparent that cancer patients receiving anti-VEGF therapy (e.g. Avastin) exhibit preeclampsia-like symptoms [7]. Compelling clinical studies showed that serum levels of sFlt-1 and placenta growth factor (PlGF) gave the highest strength of association with the clinical manifestation of preeclampsia [8–10]. It is almost certain that the loss of VEGF activity appears to be responsible for the clinical signs of preeclampsia. Soluble Flt-1 acts as a potent inhibitor of VEGF- and PlGF-mediated biological activities by sequestering these ligands and by preventing ligand-receptor dimerisation with full-length VEGF receptors (Fig. 1). It has been proposed that reducing the circulating levels of free sFlt-1 below a certain threshold in women with preeclampsia could alleviate the clinical signs of the condition [11], as below a critical threshold, sFlt-1 fails to induce hypertension or proteinurea in mice [12]. Indeed, women with fetal growth-restriction can have elevated sFlt-1 levels without hypertension or proteinurea [13].

Dysregulation of another homeostatic factor has also been reported. Transforming growth factor (TGF)-β is an anti-inflammatory growth factor [14] that activates eNOS [15]. Neutralisation of TGF-β leads to endothelial dysfunction characterised by impaired endothelium-mediated vasodilatation and elevated expression of surface adhesion molecules, resulting in increased leukocyte adhesion [16]. Proteolytic cleavage of the extracellular domain of endoglin, a transmembrane co-receptor for TGF-β1 and TGF-β3, generates soluble endoglin (sEng) that functions to limit the activity of TGF-β signalling and eNOS [17,18] (Fig. 1). sEng acts synergistically with sFlt-1 to induce endothelial dysfunction and HELLP syndrome in pregnant rats [15]. sEng is elevated in the serum of preeclamptic women many weeks prior to the clinical onset of the disease [9]. Increase in circulating sEng has direct negative effects on endothelial health *in vivo* [16] and reduces the number of regulatory T cells observed in the systemic circulation of preeclamptic women [19]. Little is known about the molecular mechanisms regulating sEng release except that cytokines [20] and AT_1 receptor autoantibodies [21] increase both sFlt-1 and sEng and that the stress-responsive heme oxygenase-1 (HO-1) inhibits their release.

New mechanisms of preeclampsia

Smoking during pregnancy is highly associated with spontaneous abortion; stillbirth, preterm labor, fetal growth restriction and placental abruption, but the incidence of preeclampsia is reduced by a third in smokers [22], compared to snuff (smokeless tobacco) users [23]. This indicates that it is a combustible product of tobacco that confers the protection. Carbon monoxide (CO) is generated endogenously in cells by the catalytic breakdown of free heme by the enzyme hemeoxygenases (HO). The stress-responsive HO-1 isozyme confers cytoprotection against tissue and cellular injury [24–26] and is protective against ischemia-reperfusion injury [27–30]. Ahmed et al. demonstrated that the loss of HO activity promoted placental damage induced by TNFα, which could be prevented by increasing HO activity by hemin [31] (Fig. 2). This led them to the proposal that a lack of HO/CO

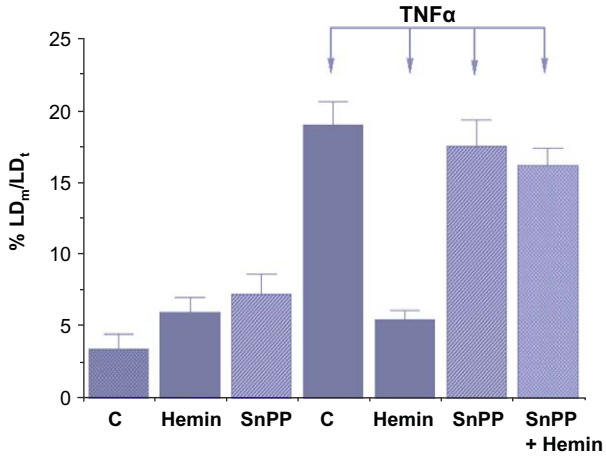

Fig. 2. The loss of HO activity promotes TNFα-induced placental damage. Villous fragments were incubated with either vehicle (C), hemin (activator of HO), SnPP (inhibitor of HO), and TNFα (50 ng/ml) alone or TNFα added after 2 hours pre-incubation with hemin or SnPP. In the presence of SnPP, the protective effect of hemin on TNFα-induced damage was totally abolished. The cytotoxicity index is expressed as a percentage ratio of lactate dehydrogenase (LD) leakage into media / LD in tissue (LDm/LDt). Data represents the mean (±sem) of eight separated experiments performed in duplicate.

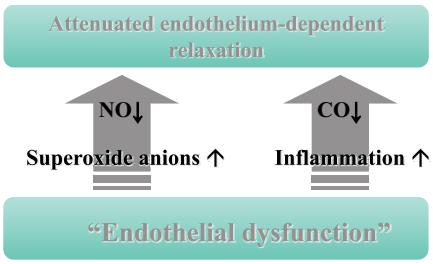

Fig. 3. A new model of endothelial dysfunction in preeclampsia. There is increasing evidence to support that it is the loss of HO/CO activity, which leads to exacerbated inflammation as well as the loss of nitric oxide (NO) bioavailability that give rise to the preeclamptic phenotype.

activity predisposes women during pregnancy to preeclampsia and that it was the HO/CO pathway that was responsible for resolution of the inflammation associated with pregnancy. All diseases have an inflammatory component and preeclampsia is no exception. However, it is not increased in inflammation *per se* that causes preeclampsia, but rather the loss of HO/CO activity, which leads to exacerbated inflammation (Fig. 3). Smokers are known to have reduced circulating sFlt-1 and increased placental growth factor [9]. Cigarette smoke extract induces HO-1 expression in trophoblasts [32] and decreases sFlt-1 release from placental villous explants without altering placental apoptotic status [33]. CO treatment has been shown to enhance HO-1 expression [34] and HO-1 mRNA is decreased in the blood of preeclamptic women at term [35]. Furthermore, women with preeclampsia have significantly decreased CO concentrations in their exhaled breath compared to those with healthy pregnancies indicating a decreased HO activity [36,37].

The hypothesis that HO-1 mediated CO release protects against preeclampsia is strengthen by mounting evidence that this stress response gene and its gaseous product confer protection during pregnancy [20,21,31,38]. The most compelling evidence for this theory comes from a recent study using fetal placental cells (chorionic villous sampling, CVS) from women at 11 weeks gestation. Farina and colleagues showed that the expression of HO-1 mRNA decreased in CVS from women who went on to develop preeclampsia [39]. This data opens up the possibility that this very early decrease in HO-1 could lead, at least in part, to the elevated anti-angiogenic factors seen in preeclamptic women later in pregnancy. Indeed, both CO and over-expression of HO-1 inhibit the production of sFlt-1 and sEng [20]. Furthermore, HO-1 null mice have systemic endothelial damage and greatly elevated circulating sEng [20]. A recent publication showed that the angiotensin receptor agonistic auto-antibody stimulates sEng *in vivo* by upregulation of TNF-α and this upregulation can be prevented by induction of HO-1 using hemin [21]. The HO enzyme system generates three molecules (Biliverdin, Fe^{2+} and CO), which are unique in that they all have biological activity. Biliverdin is an antioxidant, which is rapidly reduced by biliverdin reductase to bilirubin, another potent antioxidant. The interactive role of these in preeclampsia still needs to be evaluated.

New intervention

A cheap, effective and safe therapy for prevention of preeclampsia complications is urgently needed. Low-dose aspirin is the only prophylactic therapy for primary prevention, but only mildly reduces the risk of future preeclampsia in high-risk women [40]. Agents capable of substituting for the deficiency or inducing the activity of the HO system and/or reducing the elevated sFlt-1/sEng may have therapeutic potential for alleviating the severity of preeclampsia and in turn prolong pregnancy in early-onset disease, so reducing the complication burden for both mother and neonate.

Although statins are contraindicated in pregnancy, a recent observational study on the use of statins in the first trimester of pregnancy looked at 288 women and found no adverse effects [41]. Statins inhibit cytokine-mediated release of sFlt-1 in cultured placental explants [20] and also the level of this anti-angiogenic factor in pregnant mice (unpublished data). Statins have anti-inflammatory properties and increase activity of the thioredoxin [42], SOD [43] and glutathione peroxidase [44] systems. Statins also improve factors that are compromised in preeclampsia such as NO bioavailability, VEGF and endothelial progenitor cells [20,45]. The world's first randomised placebo-controlled trial, **StAmP** (**St**atins to **Am**eliorate Early Onset **P**re-eclampsia) for the use of statins in early-onset preeclampsia is underway and its outcome will inform obstetricians whether use of statins in preeclampsia is viable.

Women destined to develop early onset preeclampsia could be offered preventative statin or CO therapy. It may be necessary to give statins and CO as a combination therapy for the best outcome; this is something that can only be evaluated after the StAmP trial.

This review has outlined the evidence supporting the notion that activation of the heme oxygenase/CO pathway by statins could lead to alleviation of the signs of preeclampsia and to prolongation of affected pregnancies. This would seriously improve outcomes for mothers and babies globally and reduce the lifelong negative health impacts of preeclampsia. If StAmP is successful, the dawn of an era of cheap, widely available therapy against preeclampsia may occur that could reduce worldwide maternal and infant mortality associated with preeclampsia.

Acknowledgements

The author appreciates the collegiality and the dedication of the past and present members of his laboratory.

Conflict of Interest Statement

There are no conflicts of interest.

Funding

The author's work described in this review was supported by grants from the Medical Research Council (G0601295 and G0700288) and British Heart Foundation (RG/09/001/25940).

References

[1] Heitzer T, Schlinzig T, Krohn K, Meinertz T, Munzel T. Endothelial dysfunction, oxidative stress, and risk of cardiovascular events in patients with coronary artery disease. Circulation 2001;104:2673–8.
[2] Ferrara N. Vascular endothelial growth factor: basic science and clinical progress. Endocr Rev 2004;25:581–611.
[3] Ahmad S, Hewett PW, Wang P, Al-Ani B, Cudmore M, Fujisawa T, et al. Direct evidence for endothelial vascular endothelial growth factor receptor-1 function in nitric oxide-mediated angiogenesis. Circ Res 2006;99:715–22.
[4] Xenos ES, Stevens SL, Freeman MB, Cassada DC, Goldman MH. Nitric oxide mediates the effect of fluvastatin on intercellular adhesion molecule-1 and platelet endothelial cell adhesion molecule-1 expression on human endothelial cells. Ann Vasc Surg 2005;19:386–92.
[5] Ahmed A. Heparin-binding angiogenic growth factors in pregnancy. Trophoblast Res 1997;10:215–58.
[6] Maynard SE, Min JY, Merchan J, Lim KH, Li J, Mondal S, et al. Excess placental soluble fms-like tyrosine kinase 1 (sFlt1) may contribute to endothelial dysfunction, hypertension, and proteinuria in preeclampsia. J Clin Invest 2003;111:649–58.
[7] Kabbinavar F, Hurwitz HI, Fehrenbacher L, Meropol NJ, Novotny WF, Lieberman G, et al. Phase II, randomized trial comparing bevacizumab plus fluorouracil (FU)/leucovorin (LV) with FU/LV alone in patients with metastatic colorectal cancer. J Clin Oncol 2003;21:60–5.
[8] Levine RJ, Maynard SE, Qian C, Lim KH, England LJ, Yu KF, et al. Circulating angiogenic factors and the risk of preeclampsia. N Engl J Med 2004;350:672–83.

[9] Levine RJ, Lam C, Qian C, Yu KF, Maynard SE, Sachs BP, et al. Soluble endoglin and other circulating antiangiogenic factors in preeclampsia. N Engl J Med 2006;355:992–1005.
[10] Noori M, Donald AE, Angelakopoulou A, Hingorani AD, Williams DJ. Prospective study of placental angiogenic factors and maternal vascular function before and after preeclampsia and gestational hypertension. Circulation 2010;122:478–87.
[11] Ahmed A, Cudmore MJ. Can the biology of VEGF and haem oxygenases help solve pre-eclampsia? Biochem Soc Trans 2009;37:1237–42.
[12] Bergmann A, Ahmad S, Cudmore M, Gruber AD, Wittschen P, Lindenmaier W, et al. Reduction of circulating soluble Flt-1 alleviates preeclampsia-like symptoms in a mouse model. J Cell Mol Med 2010;14:1857–67.
[13] Wallner W, Sengenberger R, Strick R, Strissel PL, Meurer B, Beckmann MW, et al. Angiogenic growth factors in maternal and fetal serum in pregnancies complicated by intrauterine growth restriction. Clin Sci (Lond) 2007;112:51–7.
[14] Robertson AK, Rudling M, Zhou X, Gorelik L, Flavell RA, Hansson GK. Disruption of TGF-beta signaling in T cells accelerates atherosclerosis. J Clin Invest 2003; 112:1342–50.
[15] Venkatesha S, Toporsian M, Lam C, Hanai J, Mammoto T, Kim YM, et al. Soluble endoglin contributes to the pathogenesis of preeclampsia. Nat Med 2006;12: 642–9.
[16] Walshe TE, Saint-Geniez M, Maharaj AS, Sekiyama E, Maldonado AE, D'Amore PA. TGF-beta is required for vascular barrier function, endothelial survival and homeostasis of the adult microvasculature. PLoS ONE 2009;4: e5149.
[17] Jerkic M, Rivas-Elena JV, Prieto M, Carron R, Sanz-Rodriguez F, Perez-Barriocanal F, et al. Endoglin regulates nitric oxide-dependent vasodilatation. FASEB J 2004;18:609–11.
[18] Toporsian M, Gros R, Kabir MG, Vera S, Govindaraju K, Eidelman DH, et al. A role for endoglin in coupling eNOS activity and regulating vascular tone revealed in hereditary hemorrhagic telangiectasia. Circ Res 2005;96:684–92.
[19] Santner-Nanan B, Peek MJ, Khanam R, Richarts L, Zhu E, Fazekas de St Groth B, et al. Systemic increase in the ratio between Foxp3+ and IL-17-producing CD4+ T cells in healthy pregnancy but not in preeclampsia. J Immunol 2009;183: 7023–30.
[20] Cudmore M, Ahmad S, Al-Ani B, Fujisawa T, Coxall H, Chudasama K, et al. Negative regulation of soluble Flt-1 and soluble endoglin release by heme oxygenase-1. Circulation 2007;115:1789–97.
[21] Zhou CC, Irani RA, Zhang Y, Blackwell SC, Mi T, Wen J, et al. Angiotensin receptor agonistic autoantibody-mediated tumor necrosis factor-alpha induction contributes to increased soluble endoglin production in preeclampsia. Circulation 2010;121:436–44.
[22] Conde-Agudelo A, Althabe F, Belizan JM, Kafury-Goeta AC. Cigarette smoking during pregnancy and risk of preeclampsia: a systematic review. Am J Obstet Gynecol 1999;181:1026–35.
[23] England LJ, Levine RJ, Mills JL, Klebanoff MA, Yu KF, Cnattingius S. Adverse pregnancy outcomes in snuff users. Am J Obstet Gynecol 2003;189:939–43.
[24] Otterbein LE, Kolls JK, Mantell LL, Cook JL, Alam J, Choi AM. Exogenous administration of heme oxygenase-1 by gene transfer provides protection against hyperoxia-induced lung injury. J Clin Invest 1999;103:1047–54.
[25] Keyse SM, Tyrrell RM. Heme oxygenase is the major 32-kDa stress protein induced in human skin fibroblasts by UVA radiation, hydrogen peroxide, and sodium arsenite. Proc Natl Acad Sci U S A 1989;86:99–103.
[26] Duckers HJ, Boehm M, True AL, Yet SF, San H, Park JL, et al. Heme oxygenase-1 protects against vascular constriction and proliferation. Nat Med 2001;7:693–8.
[27] Melo LG, Agrawal R, Zhang L, Rezvani M, Mangi AA, Ehsan A, et al. Gene therapy strategy for long-term myocardial protection using adeno-associated virus-mediated delivery of heme oxygenase gene. Circulation 2002;105:602–7.
[28] Katori M, Anselmo DM, Busuttil RW, Kupiec-Weglinski JW. A novel strategy against ischemia and reperfusion injury: cytoprotection with heme oxygenase system. Transpl Immunol 2002;9:227–33.
[29] Zhang X, Shan P, Jiang D, Noble PW, Abraham NG, Kappas A, et al. Small interfering RNA targeting heme oxygenase-1 enhances ischemia-reperfusion-induced lung apoptosis. J Biol Chem 2004;279:10677–84.
[30] Tsuchihashi S, Fondevila C, Kupiec-Weglinski JW. Heme oxygenase system in ischemia and reperfusion injury. Ann Transplant 2004;9:84–7.
[31] Ahmed A, Rahman M, Zhang X, Acevedo CH, Nijjar S, Rushton I, et al. Induction of placental heme oxygenase-1 is protective against TNFalpha-induced cytotoxicity and promotes vessel relaxation. Mol Med 2000;6:391–409.
[32] Sidle EH, Casselman R, Smith GN. Effect of cigarette smoke on placental antioxidant enzyme expression. Am J Physiol Regul Integr Comp Physiol 2007;293:R754–8.
[33] Mehendale R, Hibbard J, Fazleabas A, Leach R. Placental angiogenesis markers sFlt-1 and PlGF: response to cigarette smoke. Am J Obstet Gynecol 2007;197: 363.e1–363.e5.
[34] Thom SR, Fisher D, Xu YA, Notarfrancesco K, Ischiropoulos H. Adaptive responses and apoptosis in endothelial cells exposed to carbon monoxide. Proc Natl Acad Sci U S A 2000;97:1305–10.
[35] Nakamura M, Sekizawa A, Purwosunu Y, Okazaki S, Farina A, Wibowo N, et al. Cellular mRNA expressions of anti-oxidant factors in the blood of preeclamptic women. Prenat Diagn 2009;29:691–6.
[36] Baum M, Schiff E, Kreiser D, Dennery PA, Stevenson DK, Rosenthal T, et al. End-tidal carbon monoxide measurements in women with pregnancy-induced hypertension and preeclampsia. Am J Obstet Gynecol 2000;183:900–3.
[37] Kreiser D, Baum M, Seidman DS, Fanaroff A, Shah D, Hendler I, et al. End tidal carbon monoxide levels are lower in women with gestational hypertension and pre-eclampsia. J Perinatol 2004;24:213–7.
[38] Acevedo CH, Ahmed A. Hemeoxygenase-1 inhibits human myometrial contractility via carbon monoxide and is upregulated by progesterone during pregnancy. J Clin Invest 1998;101:949–55.
[39] Farina A, Sekizawa A, De Sanctis P, Purwosunu Y, Okai T, Cha DH, et al. Gene expression in chorionic villous samples at 11 weeks' gestation from women destined to develop preeclampsia. Prenat Diagn 2008;28:956–61.
[40] Bates SM, Greer IA, Hirsh J, Ginsberg JS. Use of antithrombotic agents during pregnancy: the Seventh ACCP Conference on Antithrombotic and Thrombolytic Therapy. Chest 2004;126:627S–644S.
[41] Ofori B, Rey E, Berard A. Risk of congenital anomalies in pregnant users of statin drugs. Br J Clin Pharmacol 2007;64:496–509.
[42] Haendeler J, Hoffmann J, Zeiher AM, Dimmeler S. Antioxidant effects of statins via S-nitrosylation and activation of thioredoxin in endothelial cells: a novel vasculoprotective function of statins. Circulation 2004;110:856–61.
[43] Landmesser U, Bahlmann F, Mueller M, Spiekermann S, Kirchhoff N, Schulz S, et al. Simvastatin versus ezetimibe: pleiotropic and lipid-lowering effects on endothelial function in humans. Circulation 2005;111:2356–63.
[44] Molcanyiova A, Stancakova A, Javorsky M, Tkac I. Beneficial effect of simvastatin treatment on LDL oxidation and antioxidant protection is more pronounced in combined hyperlipidemia than in hypercholesterolemia. Pharmacol Res 2006; 54:203–7.
[45] Urbich C, Dimmeler S. Risk factors for coronary artery disease, circulating endothelial progenitor cells, and the role of HMG-CoA reductase inhibitors. Kidney Int 2005;67:1672–6.